Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 12932–12936, December 1994
Immunology

Interferon γ-independent effects of interleukin 12 administered during acute or established infection due to *Leishmania major*

(cell-mediated immunity/interleukin 4/leishmaniasis/cytokines)

ZHI-EN WANG*†, SHICHUN ZHENG*†, DAVID B. CORRY*, DYANA K. DALTON‡, ROBERT A. SEDER§, STEVEN L. REINER*, AND RICHARD M. LOCKSLEY*

*Departments of Medicine and Microbiology/Immunology, University of California, San Francisco, CA 94143; ‡Systemix, Inc., Palo Alto, CA 94304; and §Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892

Communicated by Elizabeth H. Blackburn, September 26, 1994

ABSTRACT **Interleukin 12 (IL-12) is a powerful stimulus for the growth of activated T and natural killer cells, their generation of interferon γ (IFN-γ), and the differentiation of T helper type 1 (T_{h1}) effector cells from naive precursors *in vitro*. These activities are consistent with the capacity of exogenous IL-12 to heal otherwise susceptible BALB/c mice infected with the intramacrophage parasite *Leishmania major*. Using this characterized model of CD4 cell subset differentiation, we examined the immunologic effects of IL-12 administered either at the time of infection, when naive T cells are primed, or after 14 days of infection, by which time $CD4^+$ subset differentiation has occurred. Given with the inoculation of parasites, IL-12 induced IFN-γ and IL-10 and markedly suppressed IL-4. Effects on IL-10 and IL-4 were comparable in mice with homozygous disruption of the IFN-γ gene ($IFN\text{-}\gamma^{0/0}$), and suppression of IL-4 was unchanged by administration of neutralizing anti-IL-10 antibody. Induction of IFN-γ and IL-10 mRNA by IL-12 also occurred in infected SCID mice. Given after day 14 of infection, however, IL-12 not only induced IFN-γ and IL-10 but also induced IL-4 in normal and $IFN\text{-}\gamma^{0/0}$ mice. These data demonstrate direct effects of IL-12 independent of IFN-γ, IL-10, and IL-4 and demonstrate that the ineffectiveness of IL-12 administered following infection with *L. major* correlates with resistance of differentiated T_{h2} cells to the IL-4-suppressing activity of IL-12.**

Experimental infection of mice with *Leishmania major* is a well-characterized model for the differentiation of $CD4^+$ effector cell populations *in vivo* (1, 2). Inbred strains capable of controlling infection develop *Leishmania*-specific T helper type 1 (T_{h1}) cells that provide macrophage-activating cytokines required for restricting the spread of the intracellular amastigotes. Interferon γ (IFN-γ) is particularly important among these T_{h1}-derived cytokines; animals deficient in IFN-γ are unable to restrict growth of the parasite and develop progressive infection (3). Genetically susceptible mice, such as BALB/c, develop ineffective T_{h2} responses to *L. major* and likewise fail to control infection (2). The presence of the counter-regulatory cytokines interleukin 4 (IL-4) and IL-10 presumably accounts for the failure of exogenously provided IFN-γ to overcome genetically programmed T_{h2} cell development or favorably influence the course of disease (4).

IL-12 is a heterodimeric cytokine produced by macrophages and B-cell lines that has been implicated in T_{h1} cell development *in vitro* (5, 6). IL-12 is a growth factor and a potent stimulus for IFN-γ production from activated T lymphocytes and natural killer (NK) cells (7, 8). In contrast to IFN-γ, IL-12 given at the time of inoculation of *L. major* was successful in protecting BALB/c mice from progressive disease (9, 10). Exogenous IL-12 bypasses the capacity of the promastigotes to evade IL-12 induction during the establishment of natural infection (11). When given later in infection, however, IL-12 was unable to reverse the course of infection in BALB/c mice (10), suggesting some differential effect of this cytokine when given during priming or fully developed immune responses. We have used the murine model of *L. major* infection to demonstrate the marked capacity of IL-12 to suppress IL-4 when given at the time of inoculation of organisms and the loss of this capacity when IL-12 is given 2 weeks after the establishment of the immune response. Further, these responses were independent of the induction of IFN-γ, as demonstrated for mice with disruption of the IFN-γ gene.

MATERIALS AND METHODS

Animals and Parasites. Mice with targeted homozygous disruption of the IFN-γ gene ($IFN\text{-}\gamma^{0/0}$) were developed and screened as described (12), backcrossed seven generations onto the C57BL/6 and BALB/c backgrounds, and maintained along with heterozygous littermates ($IFN\text{-}\gamma^{+/0}$) in the University of California, San Francisco, Transgenic Facility. BALB/c, C57BL/6, and C.B-17/*scid* (SCID, severe combined immunodeficiency) mice were purchased from The Jackson Laboratory. Groups of four to six mice were infected in the hind footpads with 10^6 stationary-phase promastigotes of *L. major* (strain WHOM/IR/−/173) as described (2). Designated groups of mice received the following special reagents: recombinant murine IL-12, prepared as described (13), intraperitoneally as 1 μg in 0.1 ml of phosphate-buffered saline (PBS) with 1% syngeneic mouse serum for four consecutive days starting either at the same time or 14 days following inoculation of the parasites; 1 mg of anti-IL-4 monoclonal antibody (mAb) 11B11 intraperitoneally at the time of infection, to induce a healer phenotype (4); 2 mg of neutralizing anti-IL-10 mAb JES5-2A5 (rat IgG1; ref. 14) intraperitoneally at the time of inoculation of the parasites; 1 mg of anti-CD4 mAb GK1.5 for two consecutive days prior to harvest of lymph node tissues; or 1 mg of neutralizing anti-IFN-γ mAb XMG1.2 at the time of inoculation of the organisms. An additional group of uninfected mice received 800 μg of goat anti-mouse IgD mAb (kindly provided by F. Finkelman, Uniformed Services University of the Health Sciences, Bethesda, MD) intravenously as described (13).

Collection of Lymph Node Tissues. At designated times after infection, mice were killed and the popliteal lymph nodes were collected. Mice treated with anti-IgD had spleens col-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: IFN-γ, interferon-γ; IL, interleukin; HPRT, hypoxanthine phosphoribosyltransferase; NK, natural killer; mAb, monoclonal antibody.

†Z.-E.W. and S.Z. contributed equally to this paper.

lected on day 5 after injection. Half of the tissues were flash frozen in liquid nitrogen, pulverized to a fine powder, and placed in RNAzol (Biotecx Laboratories, Houston) for preparation of total RNA. The remaining specimens were teased to single-cell suspensions in iced PBS, washed twice, and suspended at 10^7 cells per ml in RPMI 1640 with 5% fetal bovine serum (FBS; HyClone) and antibiotics.

Analysis of Cytokine Transcripts. RNA was reverse transcribed with Moloney murine leukemia virus reverse transcriptase (GIBCO/BRL) and random hexamer primers (Promega) Madison (15). PCR was performed with a multiple-cytokine competitor construct (15). The construct contains sequence specific for the constitutively expressed gene encoding hypoxanthine phosphoribosyltransferase (HPRT), allowing adjustments of input cDNA to comparable amounts of HPRT expression. Adjusted cDNAs were then amplified with cytokine-specific primers (11, 15) and the products were separated by electrophoresis in an ethidium-stained 2.5% agarose gel, thus allowing discrimination of the larger competitor construct from the wild-type cDNAs, which migrate further in the gel. Photographic negative of the gels were scanned with an imaging densitometer (Bio-Rad) to quantitate band intensities and confirm visual interpretations.

ELISPOT Assays. Single-cell lymph node suspensions were distributed in duplicate aliquots (10^6 cells in 100 μl of RPMI 1640 with 5% FBS and antibiotics) to 96-well plates (Immulon II; Dynatech) that had been precoated with either mAb BVD4-1D11.2 against IL-4 or mAb R46A2 against IFN-γ. Serial 3-fold dilutions were prepared and the plates were incubated undisturbed for 8 hr at 37°C. Wells were washed with PBS to remove cells and incubated with biotinylated secondary antibodies against IL-4 (BVD6-24G.2) or IFN-γ (XMG-6), respectively. After 1 hr, wells were washed and incubated for 1 hr with 100 μl of streptavidin-conjugated alkaline phosphatase (Jackson ImmunoResearch) in PBS with 0.5% Tween 20 and 5% FBS. Color was developed with 5-bromo-4-chloro-3-indolyl phosphate in 0.1 M 2-amino-2-methyl-1-propanol buffer (Sigma) suspended in agarose (SeaPlaque; FMC); after solidification of the agar, individual blue spots were counted by inverted microscopy (3).

Antibody Isotypes. Total serum IgE was quantitated by a double mAb-based sandwich ELISA with antibody B.IE.3 as a primary antibody and biotinylated EM-95 as a detecting antibody, as described (3). Results were quantitated by comparison to a standard control (Sigma).

RESULTS

Effect of IL-12 Given During the Acute Immune Response to *L. major*. Mice given recombinant IL-12 for the first 5 days of infection were protected from progressive disease due to *L. major* (9, 10). To assess the immune response, groups of mice inoculated with *L. major* were given either 1 μg of IL-12 simultaneously and then daily for four consecutive days or a saline control and killed on the fifth day to assess the immunologic effects. Mice with homozygous disruption of the IFN-γ gene (IFN-$\gamma^{0/0}$) and their heterozygous littermates were examined concurrently to assess the effects of endogenous IFN-γ on IL-12-mediated events. Compared with uninfected mice, infection induced transcripts for IFN-γ, IL-4, IL-5, IL-10, and IL-13 in the draining lymph node cells (Fig. 1), as previously noted (11). Except for the absence of IFN-γ transcripts, similar results occurred in the IFN-$\gamma^{0/0}$ mice. Treatment with IL-12 during this period resulted in enhancement of IFN-γ and IL-10 transcripts and complete inhibition of IL-4 transcripts. There was little effect on IL-5 or IL-13. The enhancement of IL-10 and abrogation of IL-4 transcripts were comparable in IFN-$\gamma^{0/0}$ mice treated with IL-12, demonstrating that these effects are independent of IFN-γ. Similar results were seen following administration of

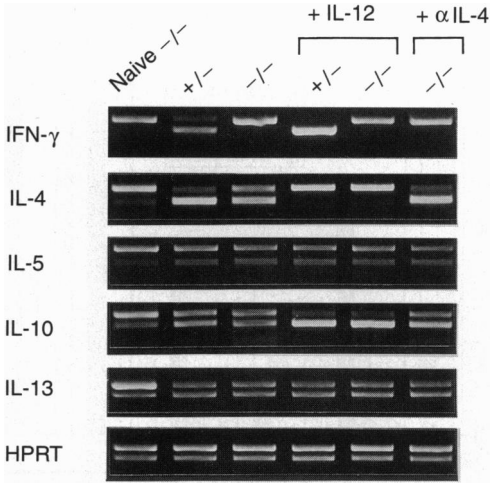

FIG. 1. Cytokine mRNA expression 5 days after infection with *L. major*. Groups of four or five BALB/c mice with homozygous (−/−) or heterozygous (+/−) disruption of the IFN-γ gene were left uninfected (Naive −/−), infected with *L. major*, or infected and treated daily with IL-12 (+ IL-12) for 4 days or once at the time of infection with anti-IL-4 mAb (+ αIL-4). Popliteal lymph node cells were harvested 1 day after the final IL-12 dose, mRNA was extracted and reverse transcribed, and, after standardization of HPRT transcript levels, the levels of the designated cytokine mRNAs were determined by coamplification by PCR in the presence of a cytokine competitor construct. After separation in an ethidium-stained agarose gel, the authentic wild-type transcripts, whose intensity increases as they out-compete the competitor, migrate farther in the gel (lower bands) than the larger competitor (upper bands). Data represent one of three comparable experiments.

neutralizing mAb for IFN-γ to infected wild-type BALB/c and C57BL/6 mice; induction of IL-10 and suppression of IL-4 by IL-12 remained unchanged (data not shown). Treatment of infected IFN-$\gamma^{0/0}$ mice with anti-IL-4, another intervention capable of reversing the susceptibility phenotype in BALB/c mice (4), had little effect on IL-4 or IL-10 mRNA levels (Fig. 1). Although results are shown for BALB/c mice in Fig. 1, results were comparable for animals backcrossed onto the C57BL/6 background (data not shown). Treatment with IL-12 did not affect the eventual fatal course of disease in IFN-$\gamma^{0/0}$ mice.

ELISPOT assays of freshly isolated lymph node cells were used to assess the spontaneous production of secreted IFN-γ and IL-4 in these same groups of mice (Fig. 2). In accord with the mRNA analysis, IL-12 treatment increased the number of IFN-γ-secreting cells in IFN-$\gamma^{+/0}$ mice ($P < 0.05$) and markedly decreased the number of IL-4 secreting cells in both IFN-$\gamma^{+/0}$ and IFN-$\gamma^{0/0}$ mice ($P < 0.05$).

Administration of depleting doses of anti-CD4 mAb for 48 hr prior to harvesting of lymph node tissues resulted in attenuation, but not complete abrogation, of IFN-γ and IL-10 transcripts induced by IL-12 at this early period after infection (data not shown). These effects of IL-12 on non-T cells were confirmed in SCID mice infected with *L. major*; induction of both IFN-γ and IL-10 by IL-12 was readily demonstrated 5 days after infection (Fig. 3). No IL-4 transcripts were demonstrable in SCID mice, suggesting that suppression of IL-4 does not mediate the enhancement of IFN-γ and IL-10 mRNA.

It is possible that enhanced levels of IL-10 mediate the suppression of IL-4 by IL-12, perhaps through the ability of IL-10 to impair IL-2 production (16). Neither IFN-$\gamma^{+/0}$ nor IFN-$\gamma^{0/0}$ mice given neutralizing amounts of anti-IL-10 mAb during the period of IL-12 treatment, however, showed impairment in suppression of IL-4 as assessed either by mRNA (Fig. 4) or by ELISPOT assays (data not shown).

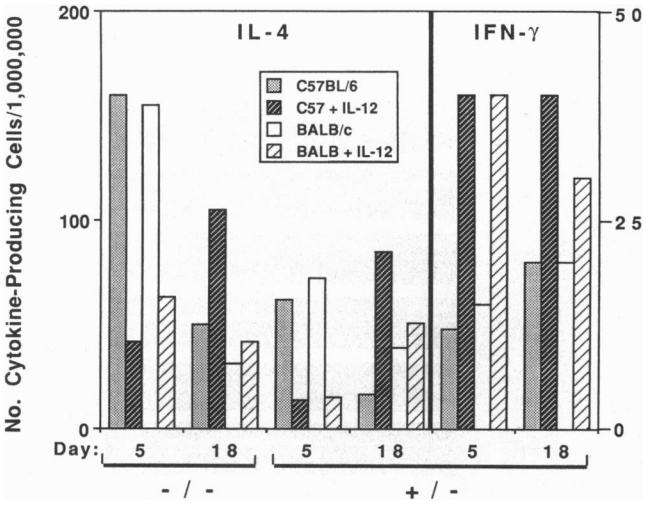

FIG. 2. ELISPOT assays for IL-4 and IFN-γ production after infection with *L. major*. Isolated popliteal lymph node cells were collected from infected IFN-$\gamma^{0/0}$ (−/−) or IFN-$\gamma^{+/0}$ (+/−) BALB/c or C57BL/6 mice on day 5 or 18 after infection. Designated mice were treated with IL-12 (+ IL-12) for 4 days before tissues were harvested. Cells were incubated in wells coated with antibodies to IL-4 or IFN-γ and, after 24 hr, were washed away to allow colorimetric detection of the captured cytokine as counted by inverted microscopy. Variation was <10% between duplicate samples. Data represent one of three comparable experiments.

Effect of IL-12 During the Acute Response to Anti-IgD. Anti-IgD is an acute and powerful inducing agent for a number of cytokines, particularly IL-4 (13). Mice with disruption of the IFN-γ gene were given anti-IgD intravenously and treated with either IL-12 (0.5 μg) or saline control daily for 5 days. Transcripts for IL-4 were significantly suppressed (over 6-fold; $P < 0.05$), and ELISPOT spot-positive cells were reduced (5-fold; $P < 0.05$) and transcripts for IL-10 were enhanced (2.5-fold; $P < 0.05$) by treatment with IL-12 (data not shown), consistent with the effects of IL-12 during acute *L. major* infection. Serum collected from similarly treated IFN-$\gamma^{0/0}$ animals on day 8 revealed suppression of total IgE levels from 43 ± 14 μg/ml in anti-IgD-treated mice to 12 ± 4 μg/ml in mice receiving IL-12 plus anti-IgD.

Effect of IL-12 on a Developed Immune Response to *L. major*. By 14 days after inoculation of the organisms, immunologic interventions have lost their capacity to ameliorate the course of disease in susceptible BALB/c mice (17),

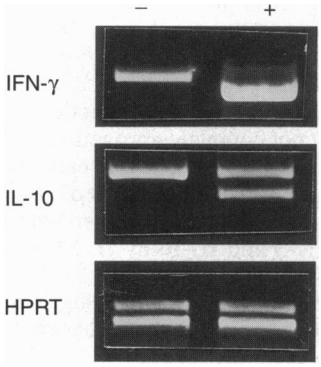

FIG. 3. IL-12 induces IFN-γ and IL-10 mRNA in infected SCID mice. Groups of five SCID mice were inoculated with *L. major* and either left untreated (−) or treated with 1 μg of IL-12 daily for four consecutive days (+). On the fifth day, popliteal lymph nodes were collected, and the mRNA was analyzed for IFN-γ and IL-10 transcripts by competitive reverse transcription–PCR as detailed in the legend to Fig. 1.

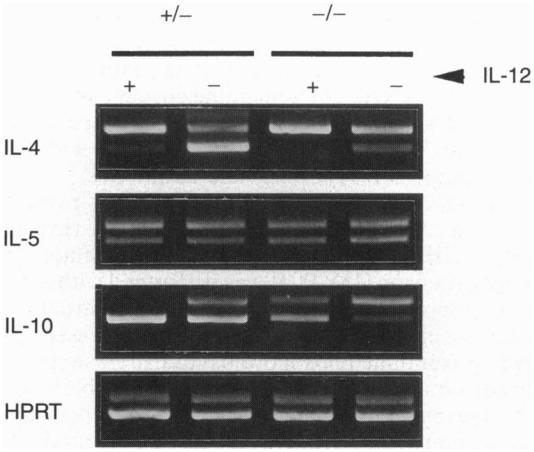

FIG. 4. Cytokine mRNA expression in mice treated with anti-IL-10 after infection with *L. major*. Groups of four BALB/c mice with heterozygous (+/−) or homozygous (−/−) disruption of the IFN-γ gene were infected with *L. major* and treated with (+) or without (−) IL-12 for 4 days. All mice received 2 mg of neutralizing anti-IL-10 mAb at the time of infection. On the fifth day, popliteal lymph node mRNA was collected and analyzed for the designated cytokine transcripts by competitive reverse transcription–PCR.

suggesting that effector lymphocytes may be less responsive than naive precursor cells. To assess this more carefully, BALB/c mice were inoculated with *L. major* and, after 14 days, treated for 4 days with either IL-12 or saline control and killed the following day to characterize the effects of IL-12 on the immune response. Although the capacity of IL-12 to induce IFN-γ and IL-10 was comparable to that in mice examined during the acute response, the capacity of IL-12 to abrogate IL-4 production was no longer present (Fig. 5). As assessed by both transcript analysis and ELISPOT assays (Fig. 2), IL-12 consistently enhanced IL-4 production when given during this later period; slight but consistent increases in IL-5 and IL-13 mRNA were also apparent. Comparable results were obtained in C57BL/6 and BALB/c IFN-$\gamma^{0/0}$ mice, suggesting no role for IFN-γ in these effects. Treatment of mice with depleting anti-CD4 antibody prior to harvesting of tissues resulted in attenuation, but not complete abroga-

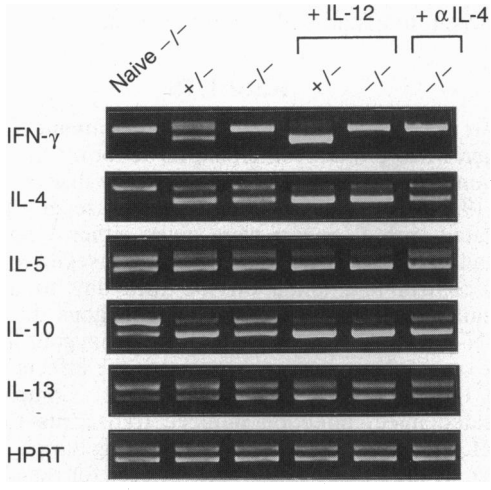

FIG. 5. Cytokine mRNA expression 18 days after infection with *L. major*. Groups of mice designated as in the legend to Fig. 1 were infected with *L. major* with or without treatment with anti-IL-4 (+ αIL-4), but treatment with four daily doses of IL-12 (+ IL-12) was not begun until 14 days after infection. Popliteal lymph node cells were collected 1 day after the last IL-12 dose and mRNA was prepared and analyzed as detailed in the legend to Fig. 1. Results represent one of three comparable experiments.

tion, of the enhancing effects of IL-12 on IFN-γ and IL-10 transcripts, although the reduction was qualitatively more marked than at the earlier time point (data not shown).

DISCUSSION

We have used experimental infection with *L. major*, a well-characterized system for analyzing $CD4^+$ effector cell differentiation, to examine the effects of exogenous IL-12 on the immune response. As shown by both mRNA- and protein-based assays, IL-12 powerfully curtailed IL-4 generation when administered at the time of inoculation of the parasites but had little effect when administered 2 weeks after established infection, despite the observation that IL-4 production is comparable at these times (11). In contrast, enhanced IFN-γ and IL-10 production occurred under both conditions. Further, as assessed in mice with homozygous disruption of the IFN-γ gene, the effects of IL-12 on IL-4 and IL-10 generation were independent of the production of IFN-γ. Similarly IFN-γ-independent effects on cytokine transcripts and production were observed with anti-IgD, an independent acute stimulus for IL-4 and IL-10 production. These data demonstrate substantial differences in the effects of IL-12 when administered during priming or during established responses that presumably underlie the reported therapeutic efficacy of IL-12 in experimental leishmaniasis (9, 10).

Prior studies examining the efficacy of IL-12 given for the first 5–7 days of infection in protecting BALB/c mice from *L. major* demonstrated suppression of IL-4 and enhancement of IFN-γ production by $CD4^+$ T cells isolated 4–5 weeks following inoculation of the organisms (9, 10). As demonstrated here, these cytokine patterns are established by the time of completion of treatment with IL-12 and include enhanced IL-10 mRNA as well. These effects of IL-12 were not seen with other interventions that enable BALB/c mice to cure this infection, such as anti-IL-4 treatment, and were independent of IFN-γ, as demonstrated with IFN-$\gamma^{0/0}$ mice. Although our prior studies demonstrated that $CD4^+$ T cells from IFN-$\gamma^{0/0}$ mice on the C57BL/6 background default to the T_{h2} phenotype following infection with *L. major* (3), the data here demonstrate the capacity of IL-12 to overcome this default pathway when administered early in both the C57BL/6 and BALB/c strains. The inefficacy of exogenous IFN-γ (4) and the potency of anti-IL-4 (4), in contrast to anti-IL-10 (17), in protecting infected BALB/c mice from *L. major* strongly suggest that the major pathway underlying the ability of exogenous IL-12 to protect BALB/c mice is the marked suppression of IL-4. The inability of anti-IL-10 to affect the suppression of IL-4 was consistent with a direct effect of IL-12 on T cells, as suggested by *in vitro* studies (5). Further, the induction of IFN-γ and IL-10 mRNA in infected SCID mice was consistent with a direct IL-4-independent effect of IL-12 on NK cells.

As previously noted (13), the use of infectious agents to investigate the immune response to IL-12 may confuse the direct effects of IL-12 with more indirect effects caused by differences in parasite survival and dissemination. As demonstrated here, however, these effects on IL-4 and IL-10 generation were also seen when IL-12 was administered at the time of intravenous inoculation of goat anti-mouse IgD, a powerful inducer of cytokine production *in vivo*. Although these data corroborate previous findings in normal mice (13), the use of IFN-$\gamma^{0/0}$ mice established that these effects were independent of the stimulation of endogenous IFN-γ production, in agreement with prior conclusions in different systems using coadministration of anti-IFN-γ antibody (13, 18). The results with anti-IgD are consistent with our findings in acute *L. major* infection and independently confirm the direct effects of IL-12 on IL-4 and IL-10 generation during acute immunologic responses.

Prior studies demonstrated the loss of efficacy of exogenous IL-12 when treatment was begun 1 week following inoculation of *L. major* (10). $CD4^+$ effector cell differentiation becomes established during this period, consistent with the inability of a number of interventions—including anti-CD4 antibody, anti-IL-4, and sublethal irradiation—to affect established effector cells. The data here suggest similar, although differential, effects of IL-12 on established immune responses, as previously noted in experiments on intestinal nematode infection (19). Most importantly, although IFN-γ and IL-10 production continued to be stimulated, IL-4 (and, to a lesser extent, IL-5) production was enhanced following administration of IL-12, and these effects were comparable in IFN-$\gamma^{+/0}$ and IFN-$\gamma^{0/0}$ mice. The mechanisms by which the regulation of IL-4 changes during the differentiation of effector T cells remain unclear. Recent data on *in vitro* systems have demonstrated differential effects of IL-12 on murine T_{h1} and T_{h2} cells, including the failure of IL-12 to serve as a costimulatory signal or upregulate the IL-2 receptor α chain on T_{h2} cells in contrast to T_{h1} cells (20–22). The recent cloning of at least one component of the IL-12 receptor (23) should permit a more direct study of the differential expression of IL-12 receptors by naive, Th_0, T_{h1}, and T_{h2} cells. The data presented here extend prior findings to *in vivo* $CD4^+$ effector cell differentiation.

Although these data are consistent with the timing requirements for IL-12 in the successful treatment of murine leishmaniasis and of murine models of melanoma metastasis (24), caution may be required before extending these findings to human studies. Thus patients with human immunodeficiency virus (HIV) infection have had restoration of cytotoxic activity and HIV-specific cell-mediated immune responses by IL-12 treatment of peripheral blood mononuclear cells *in vitro* (25, 26), and human T_{h2} clones have been induced to produce IFN-γ by incubation with IL-12 *in vitro* (27). The distribution of IL-12 receptors on T and NK cells (28) suggests that these cells are the targets affected by exogenous IL-12 administered therapeutically in *L. major* infection. Our data on SCID mice suggest that NK cells, although known to produce IFN-γ in response to IL-12 (29, 30), may also transcribe IL-10 mRNA, a finding confirmed in isolated spleen and bone marrow NK cell preparations (S. Z. and R. M. L., unpublished observations). Whether IL-12 directly affects T_{h1} development from precursor cells by suppressing autocrine IL-4 produced during priming, as suggested by *in vitro* studies (5), or suppresses IL-4 production from the $CD4^+$, $NK1.1^+$ population of cells identified as the major source of the initial IL-4 generated in response to anti-CD3 *in vivo* (31) is an important question that will require further investigation. Either mechanism might explain the powerful effect of IL-12 administered as an adjuvant to promote immune responses of the T_{h1} phenotype upon secondary challenge (32).

We thank F. Hatam and L. Stowring for maintaining parasite cultures; R. Coffman, J. Abrams, M. K., Gately, F. Finkelman, and K. Connolly for important reagents; and Genentech for the IFN-$\gamma^{0/0}$ colony. This work was supported by Grant AI26918 from the National Institutes of Health. R.M.L. is a Burroughs Wellcome Fund Scholar in Molecular Parasitology. S.Z. is a Sir Run Run Shaw Senior Scholar. D.B.C. is supported by Grant HL07185 from the National Institutes of Health.

1. Reiner, S. L., Wang, Z.-E., Hatam, F., Scott, P. & Locksley, R. M. (1993) *Science* **259**, 1457–1460.
2. Heinzel, F. P., Sadick, M. D., Mutha, S. S. & Locksley, R. M. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 7011–7015.
3. Wang, Z.-E., Reiner, S. L., Zheng, S., Dalton, D. K. & Locksley, R. M. (1994) *J. Exp. Med.* **179**, 1367–1371.
4. Sadick, M. D., Heinzel, F. P., Holaday, B. J., Pu, R. T.,

Dawkins, R. S. & Locksley, R. M. (1990) *J. Exp. Med.* **171,** 115–127.
5. Seder, R. A., Gazzinelli, R., Sher, A. & Paul, W. E. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 10188–10192.
6. Hsieh, C.-S., Macatonia, S. E., Tripp, C. S., Wolf, S. F., O'Garra, A. & Murphy, K. M. (1993) *Science* **260,** 547–549.
7. Gately, M. K., Bhupesh, B. D., Wolitzky, A. G., Quinn, P. M., Dwyer, C. M., Podlaski, F. J., Familletti, P. C., Sinigaglia, F., Chizonnite, R., Gubler, U. & Stern, A. S. (1991) *J. Immunol.* **147,** 874–882.
8. Chan, S. H., Perussia, B., Gupta, J. W., Kobayashi, M., Pospisil, M., Young, H. A., Wolf, S. F., Young, D., Clark, S. C. & Trinchieri, G. (1991) *J. Exp. Med.* **173,** 869–879.
9. Heinzel, F. P., Schoenhaut, D. S., Rerko, R. M., Rosser, L. E. & Gately, M. K. (1993) *J. Exp. Med.* **177,** 1505–1509.
10. Sypek, J. P., Chung, C. L., Mayor, S. E., Subramanyam, J. M., Goldman, S. J., Sieburth, D. S., Wolf, S. F. & Schaub, R. G. (1993) *J. Exp. Med.* **177,** 1797–1802.
11. Reiner, S. L., Zheng, S., Wang, Z.-E., Stowring, L. & Locksley, R. M. (1994) *J. Exp. Med.* **179,** 447–456.
12. Dalton, D. K., Pitts-Meek, S., Keshav, S., Figari, I. S., Bradley, A. & Stewart, T. (1993) *Science* **259,** 1739–1742.
13. Morris, S. C., Madden, K. B., Adamovicz, J. J., Gause, W. C., Hubbard, B. R., Gately, M. K. & Finkelman, F. D. (1994) *J. Immunol.* **152,** 1047–1056.
14. Abrams, J. S., Roncarolo, M.-G., Yssel, H., Andersson, U., Gleich, G. J. & Silver, J. E. (1992) *Immunol. Rev.* **127,** 5–19.
15. Reiner, S. L., Zheng, S., Corry, D. B. & Locksley, R. M. (1993) *J. Immunol. Methods* **165,** 37–46.
16. de Waal Malefyt, R., Yssel, H. & de Vries, J. (1993) *J. Immunol.* **150,** 4754–4765.
17. Coffman, R. L., Varkila, K., Scott, P. & Chatelain, R. (1991) *Immunol. Rev.* **123,** 189–205.
18. McKnight, A. J., Zimmer, G. J., Fogelman, I., Wolf, S. F. & Abbas, A. K. (1994) *J. Immunol.* **152,** 2172–2179.
19. Finkelman, F. D., Madden, K. B., Cheever, A. W., Katona, I. M., Morris, S. C., Gately, M. K., Hubbard, B. R., Gause, W. C. & Urban, J. F. (1994) *J. Exp. Med.* **179,** 1563–1572.
20. Yanagida, T., Kato, T., Igarashi, O., Inoue, T. & Nariuchi, H. (1994) *J. Immunol.* **152,** 4919–4928.
21. Germann, T., Gately, M. K., Schoenhaut, D. S., Lohoff, M., Mattner, F., Fischer, S., Jin, S.-C., Schmitt, E. & Rude, E. (1993) *Eur. J. Immunol.* **23,** 1762–1770.
22. Schmitt, E., Hoehn, P., Germann, T. & Rude, E. (1994) *Eur. J. Immunol.* **24,** 343–347.
23. Chua, A. O., Chizzonite, R., Desai, B. B., Truitt, T. P., Nunes, P., Minetti, L. J., Warrier, R. R., Presky, D. H., Levine, J. F., Gately, M. K. & Gubler, U. (1994) *J. Immunol.* **153,** 128–136.
24. Brunda, M. J., Luistro, L., Warrier, R. R., Wright, R. B., Hubbard, B. R., Murphy, M., Wolf, S. F. & Gately, M. K. (1993) *J. Exp. Med.* **178,** 1223–1230.
25. Chehimi, J., Starr, S. E., Frank, I., Rengaraju, M., Jackson, S. J., Llanes, C., Kobayashi, M., Perussia, B., Young, D., Nickbarg, E., Wolf, S. F. & Trinchieri, G. (1992) *J. Exp. Med.* **175,** 789–796.
26. Clerici, M., Lucey, D. R., Berzofsky, J. A., Pinto, L. A., Wynn, T. A., Blatt, S. P., Dolan, M. J., Hendrix, C. W., Wolf, S. F. & Shearer, G. M. (1993) *Science* **262,** 1721–1724.
27. Manetti, R., Gerosa, F., Giudici, M. G., Biagiotti, R., Parronchi, P., Piccinni, M.-P., Sanpognaro, S., Maggi, E., Romagnani, S. & Trinchieri, G. (1994) *J. Exp. Med.* **179,** 1273–1283.
28. Desai, B. B., Quinn, P. M., Wolitzky, A. G., Mongini, P. K., Chizzonite, R. & Gately, M. K. (1992) *J. Immunol.* **148,** 3125–3132.
29. Tripp, C. S., Wolf, S. F. & Unanue, E. R. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 3725–3729.
30. Gazzinelli, R. T., Hieny, S., Wynn, T. A., Wolf, S. & Sher, A. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 6115–6119.
31. Yoshimoto, T. & Paul, W. E. (1994) *J. Exp. Med.* **179,** 1285–1295.
32. Afonso, L. C., Scharton, T. M., Vieira, L. Q., Wysocka, M., Trinchieri, G. & Scott, P. (1994) *Science* **263,** 235–237.